Revisiting anti-hsp90 antibodies in systemic lupus erythematosus

Sirs,

The humoural response of systemic lupus erythematosus (SLE) patients includes antibodies directed to heat shock proteins (hsp), a group of immunogenic proteins highly preserved during the evolution of species (1). The hsp of 90kilodaltons (hsp90) is an abundant chaperone which assists in protein folding and cell signalling; it also plays a role in the cellular response to corticosteroids (2). Fifteen percent of SLE patients, all with active disease, expressed hsp90 in excess on peripheral blood mononuclear cells (3). Nevertheless, autoreactivity to hsp90 was infrequent in SLE, according to a study dated from 1991 (4). The role of anti-hsp90 antibodies as biomarkers of SLE has not been addressed for the last 15 years.

In a cross-sectional design, we evaluated anti-hsp90 levels in SLE patients and healthy controls. Diagnosis of SLE was based on the American College of Rheumatology criteria (5). Patients with SLEDAI (6) of 4 or above were classified as having active disease. Healthy volunteers matched by gender and age were utilised as controls. IgG anti-hsp90 antibodies were tested by immunoenzymatic assay (hsp90 Calbiochem - CA, USA). Briefly, 100µL of serum samples diluted in 1:100 PBS-Tween (phosphate buffer in Tween saline) were added, in pairs, to each microplate well containing hsp90, and then incubated for 90 minutes at room temperature. Plates were washed 3 times in washing solution. In the sequence, 100µL of goat IgG conjugated with peroxidase were added to each well and incubated for an additional 60 minutes. After washing and drying the plates, 100µL of the TBM (3,3',5,5'-tetramethylbenzidine) substrate were added to each well and incubated for 15 minutes at room temperature. Finally, 100µL of H_2SO_4 2.5N halting solution were added. Absorbance was read at 450 using a spectrophotometer (Microplate BIO-TEK Instruments, model EL311). Arbitrarily, the test was considered positive when optic densities (OD) were 2 standard deviations above the average of controls. To compare categoric and continuous variables, non-parametric tests and t-test were utilised.

This pilot study included 45 SLE patients (42 females, 93.3%); 22 (49%) had active disease. The control group consisted of 25 volunteers (88% females). The mean age of SLE patients and controls were 44 years and 43 years, respectively. Groups did not differ significantly as to gender and age (p>0.05). The median OD of active SLE patients (0.25; 0.10–0.68) was significantly higher as compared to inactive SLE patients (0.15; 0.06–0.5) or controls (0.15; 0.07–0.25) (p=0.001). Figure 1 shows the distribution of anti-hsp90 levels in controls and SLE patients.

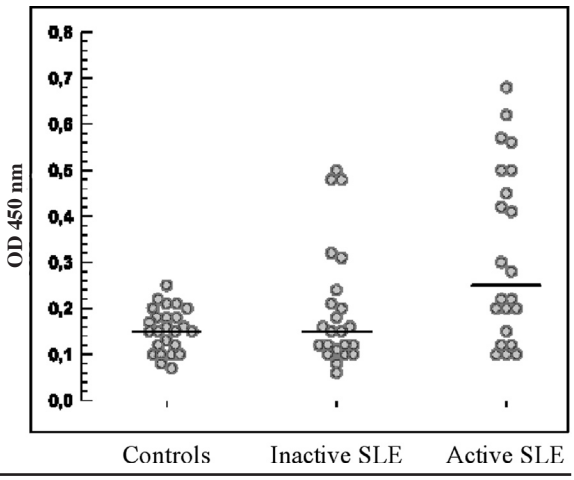

Fig. 1. Anti-hsp90 antibody levels in healthy controls and patients with inactive or active systemic lupus erythematosus (SLE). OD 450 nm: optic density at 450 nanometers.

Elevated IgG anti-hsp90 levels were detected in 36% of the 45 SLE patients (p<0.002). Half of the patients with active SLE showed positivity for anti-hsp90, compared to a 22% positivity in inactive SLE patients (p<0.05) and a null frequency in controls (p<0.001). The IgG anti-hsp90 response associated with the occurrence of anti-DNA antibodies, leukopenia and thrombocytopenia, but not with C3 or C4 depletion or changes of urinary sediment (p<0.05).

The link of anti-hsp90 antibodies with SLE is an open question. Median levels of IgG anti-hsp90 were significantly elevated in our SLE patients with active disease. A positive test was found in a third of our SLE survey. We did not stratify anti-hsp90 levels in SLE sera due to the small sample. Minota *et al*. reported anti-hsp90 antibodies by Western blot in about half of the SLE patients (7). Approximately one fourth of patients with paediatric (8) or adult (9) SLE tested positive for IgG anti-hsp90 in ELISA.

Our patients with active SLE showed significant IgG anti-hsp90 levels when compared to inactive patients or controls. In a univariate analysis, IgG anti-hsp90 positivity associated with the presence of anti-DNA antibodies, but not with nephropathy. Differently, Conroy *et al*. had documented a definite association of IgG and IgM anti-hsp90 with low C3 level and active kidney disease (9). The association of IgG anti-hsp90 response with anti-DNA antibodies and haematological SLE in our survey warrants further studies, since the number of patients with these abnormalities was rather small.

In conclusion, this pilot study showed that IgG anti-hsp90 antibodies were elevated in active SLE patients. A positive test for IgG anti-hsp90 associated with anti-DNA antibodies and haematological SLE. Given that more than a decade has passed, the role of anti-hsp90 antibodies as biomarkers of SLE could be an issue to be revisited.

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Competing interests: none declared.

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